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Insecticide Residues on Weathered Passerine Carcass Feet

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Nine brown-headed cowbirds (*Molothrus ater*) were exposed to turf sprayed with either EarthCare® (25% diazinon; 4.77 L a.i./ha) or Ortho-Klor® (12.6% chlorpyrifos; 5.21 L a.i./ha). Birds were euthanized and one foot from each bird was weathered outdoors for up to 28 days and the other foot was kept frozen until residue analysis. When compared to the unweathered feet, feet weathered for 28 days retained 43% and 37% of the diazinon and chlorpyrifos, respectively. Insecticide residues were below the level of detection (1.0 ppm) on control feet. Weathered feet may be used for determining organophosphorus insecticide exposure to birds.

Keywords: birds, chlorpyrifos, diazinon, feet, insecticides, residues, weathering

Introduction

Avian mortalities from organophosphorus insecticide exposure are investigated by federal and state wildlife personnel in the United States. The forensic investigation involves not only the recovery of the dead birds from the field but also requires laboratory confirmation of the cause of death. A carcass submitted for laboratory evaluation is subjected to pathological and anatomical examinations (Stroud and Adrian, 1996) and, if poisoning is suspected, biochemical and chemical analyses are conducted. When organophosphorus insecticide poisoning is suspected, the brain is analyzed for cholinesterase activity levels to identify the mechanism of death and chemical residue analysis is performed to identify the insecticide responsible for the death. The gastrointestinal tract or its contents are the conventional matrices used for residue analysis (Hill and Fleming, 1982).

The determination of the cause of death is contingent on the quality of the samples collected, which, in turn, is affected by how soon and how thoroughly an investigation occurs after the onset of a mortality event. Regular patrolling by wildlife personnel is prevented by the vastness of the areas subjected to insecticide applications as well as private property restrictions, which forces them to rely on public reportings. The number and quality of reportings are limited by the public's ignorance about the need for reporting wildlife mortalities and to whom the mortalities should be reported, fear of prosecution, camaraderie, procrastination, and apathy. When a mortality incident is reported to the appropriate authorities, an immediate investigation may not be possible because of the distance, terrain, weather, private property restrictions, limited resources, and other ongoing investigations. Delays in the discovery, the reporting, and the investigation of a mortality incident increase the interval between the time of mortality and carcass collection, which, in turn, increases the chances of compromising the quality of the evidence through scavenging and decomposition (Vyas, 1999; Vyas et al., 2003). Consequently, when a carcass is recovered during a field investigation, the biochemical and chemical matrices that are used to ascertain the cause of death from insecticide poisoning may be degraded and not suitable for analyzing. The loss of these matrices introduces uncertainty in determining the cause of death and reduces the found carcasses to circumstantial evidence of poisoning. State and federal wildlife personnel responding to a wildlife mortality report regard every incident as a possible legal case and, therefore, focus their efforts on recovering carcasses with analyzable matrices that can provide evidence for the cause of death. Due to the constraints on resources, field investigators may submit to the laboratory only those carcasses that retain the conventional matrices in a condition suitable for analysis.

Our objective was to determine whether insecticide residues could be detected from bird's feet that had been weathered and could then possibly provide a matrix for determining insecticide exposure.

Materials and Methods

Birds

Adult brown-headed cowbirds (*Molothrus ater*) were trapped in confusion traps from the U.S. Department of Agriculture's Beltsville Agricultural Research Center in Beltsville, Maryland, U.S.A. Birds were housed indoors for up to 2 d prior to insecticide exposure at the U.S. Geological Survey's Patuxent Wildlife

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Research Center in Laurel, Maryland, U.S.A. Husbandry followed methods described by Vyas et al. (1995).

Insecticide Exposure

Commercially purchased turf (4.6 m^2) was placed on two layers of 0.12-mm-thick plastic sheeting and sprayed with either Earth Care®, 25% diazinon a.i. (*O*,*O*-diethyl *O*-2-isopropyl-6-methyl[pyrimidine-4-yl] phosphorothioate) or Ortho-Klor®, 12.6% chlorpyrifos a.i. (*O*,*O*-diethyl-*O*-[3,5,6-trichloro-2-pyridyl] phosphorothioate). Application rates for Earth Care® (4.77 L a.i./ha) and Ortho-Klor® (5.21 L a.i./ha) followed rates described on the labels for insect control on lawns. Both insecticide applications were followed by application of 9.1 L of water as recommended on the product labels. Insecticide application was conducted by certified applicators from the Patuxent Wildlife Research Center. A bottomless, vinyl-coated wire pen (0.46 × 1.85 × 0.61 m; 1.28 cm mesh) was placed on the turf and nine cowbirds were released into the pen approximately 1 h postapplication. The low pen ceiling and a lack of perches forced the birds to be in contact with the turf. Insecticide exposure period was approximately 40 min. Birds were captured by hand from the pen, placed individually in carrying boxes and transported 1.5 km to be euthanized by CO_2 (at the Patuxent Wildlife Research Center). One bird served as a control and was not exposed to the treated turf. The turf and plastic were then incinerated. The tarsometatarsi were severed from the carcasses using scissors and each foot was individually placed in a cryovial and frozen at −23°C. Feet were kept frozen until either the weathering trial or residue analysis.

Weather Exposure

One foot from each bird was removed from the freezer and placed in a window screening wire cage (30 × 30 × 15 cm), which was placed on a wooden pallet inside a chicken wire pen (15.2 × 3.0 × 1.8 m). These pens were used for housing animals and have never been subjected to pesticide applications. Three feet per insecticide were collected after 7, 14 and 28 days of weathering and the control foot was collected on day 28. Feet were individually placed in new cryovials and frozen until residue analysis. The second foot from each bird remained frozen until analysis.

Residue Analysis

Each foot (below the distal end of the tarsometatarsus) was cut into approximately 0.6 cm pieces using scissors to facilitate chemical extraction. Samples were extracted three times with 1:1 acetone:dichloromethane, filtered, and adjusted to a 50 ml volume for analysis by gas chromatography (Belisle and Swineford, 1988). The quantitative analysis was performed using a Hewlett-Packard 5890 gas chromatograph equipped with a J&W Megabore 14% cyanopropylphenyl-86% methyl silicone capillary column and flame photometric detector. For 10% of the samples, the presence of the insecticides was confirmed on a Hewlett-Packard 5890 gas chromatograph/5970 MSD mass spectrometer (GCMS) equipped with a 50 m cross-linked methyl silicone gum column with 0.2 mm i.d. and 0.32-μ film thickness. The GCMS was linked to a 59970 ChemStation computer data system. Quality assurance was conducted through the U.S. Fish and Wildlife Service's Patuxent Analytical Control Facility in Laurel, Maryland, U.S.A. One matrix sample was spiked with 50 μg of diazinon and one matrix sample was spiked with 40 μg of chlorpyrifos and analyzed. Two method blanks were also analyzed.

Statistical Analysis

Insecticide residue concentrations were log-transformed and subjected to analysis of variance with significance established at $\alpha = 0.05$. Mean separation was conducted using the Duncan's Multiple Range Test (Freund and Littell, 1981).

Weather Data

Weather data during the foot weathering period (16 June to 14 July 1997) were obtained from the U.S. Department of Agriculture's Beltsville Agricultural Research Center's Weather Station #3 in Beltsville, Maryland, U.S.A. The station was located approximately 3.0 km from the foot weathering site.

Results and Discussion

Diazinon and chlorpyrifos levels from unweathered and weathered feet are presented in Table 1. Insecticide residues on weathered feet did not exhibit a significant decline in concentration for approximately 2 weeks for chlorpyrifos and 3 weeks for diazinon when compared to unweathered feet. Both insecticides were detected from feet weathered up to 28 days. Residue levels on the control feet were below the level of detection (<1.0 ppm). Quality assurance analysis revealed that the spike recovery was 112% and 90% for diazinon and chlorpyrifos, respectively. The two blanks contained <1.0 μg of the insecticides. Results show the utility of feet as a matrix for residue analysis when other

Table 1. *Diazinon and chlorpyrifos residues on weathered feet*

Days weathered	Diazinon (ppm)[a] geometric mean ± SD (n)	Chlorpyrifos (ppm)[a] geometric mean ± SD (n)
0[b]	6.1 ± 2.7 (9)	7.9 ± 2.9 (9)
7	8.1 ± 3.1 (3)	6.3 ± 1.9 (3)
14	3.3 ± 1.2 (3)	3.2 ± 2.3[c] (3)
28	2.6 ± 2.2[c] (3)	2.9 ± 1.4[c] (3)

[a] Control feet were weathered for 28 days and had diazinon and chlorpyrifos levels below the level of detection (1.0 ppm).
[b] Feet not placed outdoors.
[c] Significantly different from feet not placed outdoors (day 0) for their respective insecticide ($p = 0.05$).

Table 2. Weather conditions (mean ± SD) during the foot-weathering period

Days weathered	Maximum temperature (°C)	Minimum temperature (°C)	Maximum humidity (%)	Minimum humidity (%)	24 hr mean solar radiation (w/m^2)	Precipitation (mm)
7	31.1 ± 2.5	16.1 ± 3.6	101.1 ± 0.9	46.6 ± 11.3	263.6 ± 55.8	17 mm on day 2
14	31.8 ± 2.5	15.9 ± 3.3	100.9 ± 1.5	40.8 ± 11.6	273.0 ± 53.1	17 mm on day 2; 6 mm on day 10
28	31.6 ± 3.0	16.0 ± 3.1	100.7 ± 2.4	40.5 ± 15.5	259.4 ± 64.1	17 mm on day 2; 6 mm on day 10; 3 mm on day 15; 7 mm on day 16; 2 mm on day 23

tissues may not be available. Table 2 provides the weather conditions during the foot-weathering period.

Wildlife forensics laboratories may analyze insecticide residues on feet if they are available and as necessary (Frank et al., 1991; Stroud and Adrian, 1996). However, weathered feet on a decomposed or scavenged carcass may not be submitted to the laboratory by the field investigators because insecticide residues may not be expected on the feet. We focused on the feet as an indicator of insecticide exposure because for most exposure scenarios feet are likely to come into contact with pesticides—e.g., walking on treated lawn and soil (Fowle, 1972; Frank et al., 1991; Driver et al., 1991), perching on contaminated branches (Fowle 1972; Hooper et al., 1989; Henderson et al., 1994; Clark, 1997), grasping contaminated prey (Hunt et al., 1991), and wading in contaminated water (Hunt, Hooper, and Littrell, 1995).

We also focused on feet because examination of decomposed and scavenged carcasses has revealed that the feet of many carcasses remain intact even when the remainder of the carcass may not be suitable for laboratory analyses. A preliminary study was conducted in our laboratory to compare the decomposition of feet to the decomposition of carcasses. Five eastern screech owl (*Otus asio*), and six mallard (*Anas platyrhynchos*), six northern bobwhite (*Colinus virginianus*), and six brown-headed cowbird carcasses were placed in outdoor pens (2.4 × 6.1 × 1.8 m) to decompose. The vegetation in the pen was mowed to approximately 5 cm high prior to starting the preliminary study to reduce shade on the carcasses. The carcasses were placed either on their ventral or dorsal sides on bare ground between the vegetation. During this period, the mean temperature was 23°C, the mean humidity was 75%, and the total precipitation was 35 mm. All carcasses exhibited decomposition when checked after 9 d. However, the feet were intact on 15 of the 23 birds (Vyas, unpublished). During a field investigation of a suspected insecticide kill, complete decomposition (except bones, feathers, beak, and feet) of a bald eagle (*Haliaeetus leucocephalus*) carcass was determined to have occurred within 3 d (D. Patterson, personal communication). Although the carcass possessed intact feet, it was not submitted for analysis because of the absence of the conventional matrices.

Feet are also important to examine because dermal absorption of insecticides through the feet can be a significant route of exposure for birds. Fowle (1972) provided several songbird species (Order *Passeriformes*) with either perches or a floor treated with phosphamidon (dimethyl hydrogen phosphate ester with 2-chloro-*N*,*N*-diethyl-2-hydroxycrotonomide). Signs of toxicity and mortality were observed in birds within 30 min of exposure. Additionally, in the same study Fowle (1972) treated the feet of several songbird species with phosphamidon using a microsyringe and observed mortality as soon as 15 min after exposure. In another study by Hunt et al. (1991), house sparrows (*Passer domesticus*) using perches treated with fenthion (*O*,*O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl]phosphorothioate) for 30 s to 16.5 min demonstrated signs of toxicity as early as 16.5 min after introduction to the perch. Residue analyses of their plumage and skin, internal carcasses, and feet revealed the highest fenthion concentrations were found in the feet.

The possible persistence of insecticides in the feet of live birds indicates the potential for detecting residues from weathered feet. Henderson et al. (1994) exposed rock doves (*Columba livia*) to diazinon, methidathion (*O*,*O*-dimethyl hydrogen phosphorodithioate, *S*-ester with 4-(mercaptomethyl)-2-methoxy-Δ^2-1,3,4-thiadiazolin-5-one), and ethyl parathion (*O*,*O*-diethyl *O*-[*p*-nitrophenyl] phosphorothioate) either dermally via the feet or orally by gavage. Birds dermally exposed to diazinon and ethyl parathion exhibited a slower recovery (6 weeks) of plasma cholinesterase activity than the birds exposed orally (3–5 days). The authors suggested that the prolonged effects on cholinesterase activity of dermally exposed birds occurred because the insecticides could be stored under the scales of the feet and were slowly released into the blood stream.

Diazinon and chlorpyrifos were selected as representative organophosphorus insecticides because of their high toxicity to birds, their agricultural and nonagricultural (lawns, golf courses, ornamentals, residential, industrial, roadsides, range, grasslands, pastures) uses and their availability as restricted-use and over-the-counter products at the time of the study. In 2000, the U.S. Environmental Protection Agency (U.S. EPA) announced a phase-out of most indoor residential uses, indoor nonresidential uses, outdoor nonagricultural uses, and certain agricultural uses of both insecticides to mitigate ecological and human health risks by 2005 (U.S. EPA, 2000, 2001, 2002). The detection of diazinon and chlorpyrifos from our samples demonstrates the effective use of feet to determine avian exposure to organophosphorus insecticides. Insecticide levels from feet represent the chemicals on and in the feet. The residue levels from the feet do not necessary imply that the birds received a lethal dermal exposure but provide evidence about the kind of insecticide to which the bird was exposed and suggest the minimum insecticide concentration that was initially on the foot (Stroud and Adrian, 1996). However, depending on the insecticide and the findings from the field investigation, detection of certain insecticides from the feet may provide evidence to the cause of death.

While all of our insecticide-exposed birds had measurable residues on their feet, the lack of residues on weathered feet from the field does not imply that the bird was not exposed to an insecticide. The success of detecting an insecticide or its metabolites on feet depends on the method of insecticide application, the chemical concentration on the matrix with which the feet were in contact, the amount of contact with uncontaminated surfaces that may wipe the insecticide off the feet, the chemical absorption rate into the blood, and the particular insecticide. In addition, the success of detecting insecticide residues depends on the lag time between the mortality and the time of feet collection in addition to the behavior of the avian species found. The half-lives of diazinon and chlorpyrifos are affected by the weather, pH, soil organic content, and the presence of appropriate microbes. Diazinon and chlorpyrifos degrade by hydrolysis, photolysis, and microbial metabolism. The half-lives of diazinon range from 12 h to 6 mos and the half-lives of chlorpyrifos range between 1–24 wk depending on the environmental conditions and on whether the insecticide is in contact with the soil, water, or vegetation. In general, the persistence of both insecticides in the environment increases under conditions of low moisture, low temperature, and the lack of suitable microbial degraders. Diazinon is more stable at high pH, whereas chlorpyrifos is more persistent at low pH (U.S. EPA, 1999, 2000).

We excluded foot decomposition by invertebrates by placing the feet in window screening cages and we excluded insecticide degradation via soil microbes by placing the feet on wooden pallets. This design simulated instances where intact feet were found on decomposed or scavenged carcasses.

Field investigators encounter a spectrum of evidence ranging from fresh carcasses in ideal condition for laboratory analysis to scavenged and decomposed carcasses that may be of little use in determining the cause of death (Vyas et al., 2003). White and coworkers (1990) studied the survival of free-living northern bobwhites in cropland subjected to insecticide application. Despite radio-telemetry monitoring to locate the carcasses, the researchers were not able to determine the cause of mortality for any of the birds because scavenging rendered the carcasses unsuitable for necropsy and pesticide analysis. During field trials with the avicide 4-aminopyridine, Woronecki et al. (1979) could not determine the cause of death for 24 of 26 avian carcasses due to their deteriorated condition. These examples show how pesticide poisoning can remain unproven when the conventionally used matrices of measurement are not available for confirming the cause of death. Our findings provide a tool for determining insecticide exposure when the traditional matrices are not available for analysis. Carcasses previously not submitted for residue analysis because of their advanced stage of decomposition may now be salvaged for their feet. Insecticide analysis of weathered feet can raise the certainty of the cause of death depending on the complementary information collected during field investigations and the history of wildlife mortalities from a particular insecticide use.

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